Search Strategy

FILE 'USPATFULL' ENTERED AT 17:14:42 ON 10 JAN 2002

```
                E LENNERSTRAND JOHAN/IN
L1              1 S E3
                E LARDER BRENDAN/IN
L2              2 S E4 OR E5
L3          31667 S (REVERSE TRANSCRIPTASE OR RT)
L4           5034 S L3 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L5           4425 S L4 AND (ASSAY OR SCREENING)
L6              1 S L5 AND (BRDUTP OR BROMO-DEOXYURIDINE-TRIPHOSPHATE)
L7            639 S L5 AND (DRUG-RESISTAN? OR DRUG FAILURE OR CLINICAL FAILURE OR
L8            425 S L7 AND (FLUORESC? OR FLUORIMET? OR COLORIMET? OR CHEMILUMINES
L9            380 S L8 AND ANTIBOD?
L10           277 S L9 AND MUTANT?
L11            65 S L10 AND (HIGH-THROUGHPUT)
L12            35 S L10 AND (RT/AB OR REVERSE TRANSCRIPTASE/AB OR HIV-1/AB OR HUM
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FILE 'MEDLINE' ENTERED AT 17:43:01 ON 10 JAN 2002

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                E LENNERSTRAND J/AU
L13            10 S E3
L14             0 S L13 AND BRDUTP
                E LARDER B/AU
L15            93 S E3 OR E4
L16            75 S L15 AND (REVERSE TRANSCRIPTASE OR RT OR ENZYME)
L17             0 S L16 AND BRDUTP
L18            68 S L16 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L19             3 S L18 AND PY=1999
L20             9 S L18 AND PY=2000
L21             6 S L18 AND PY=1998
L22             0 S L18 AND PY=1997
L23             2 S L18 AND PY=1996
L24            35 S BRDUTP OR BROMO-DEOXYURIDINE-TRIPHOSPHATE
L25             4 S L24 AND (RT OR REVERSE TRANSCRIPTASE)
L26        138542 S (RT OR REVERSE TRANSCRIPTASE)
L27          6851 S L26 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)
L28          1117 S L27 AND (DRUG RESISTANCE OR DRUG FAILURE OR DRUG SUSCEPTIBILI
L29             5 S L28 AND (POLY-RA OR PRA)
L30             8 S L28 AND PROGNOSIS
L31           120 S L28 AND (67 OR 69 OR 70)
L32             4 S L31 AND (67 AND 69 AND 70)
L33             4 S L31 AND (INSERTION MUTATION?)
L34             3 S L33 NOT L32
L35             0 S L26 AND (ATP AND GTP AND PYROPHOSPHATE)
L36           671 S L26 AND ATP
L37            24 S L36 AND GTP
L38             0 S L37 AND PYROPHOSPHATE
L39             0 S L37 AND PPI
L40            14 S L27 AND GTP
L41            35 S L27 AND ATP
L42            93 S L26 AND (PYROPHOSPHATE OR PPI)
L43            33 S L42 AND L27
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FILE 'WPIDS' ENTERED AT 19:07:20 ON 10 JAN 2002

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                E LENNERSTRAND J/IN
L44             1 S E3
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              E LARDER B/IN
L45           2 S E3
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L1 ANSWER 1 OF 1 USPATFULL
2000:138085 Method for determining activity of reverse transcriptase.
Gronowitz, Jan-Simon, Uppsala, Sweden
Kallander, Clas, Uppsala, Sweden
Lennerstrand, Johan , Bromma, Sweden
Cavidi Tech AB, Uppsala, Sweden (non-U.S. corporation)
US 6132995 20001017
WO 9806873 19980219
APPLICATION: US 1998-51084 19980413 (9)
WO 1996-SE990 19960812 19980413 PCT 371 date 19980413 PCT 102(e) date
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for determining the activity of a nucleotide polymerizing enzyme in a sample, and use of the method for determining HIV 1 RT- and Herpes Simplex DNA-polymerase activity. The enzyme is captured by means of a nonoclonal antibody which is immobilized to a solid carrier and is capable of binding the enzyme without detrimentally effecting the enzyme activity. Contaminants and disturbing factors are removed and the nucleotide polymerization starts by the addition of a reaction solution containing a primer/template construct and nucleotides substrate, the reaction conditions being chosen such that they promote permanent association between antibody enzyme- and primer/template constructs. When necessary a nucleotide substrate, primer/template and reaction solution are washed away from the newly synthesized polymer, and the amount of nucleotide which as been incorporated into the polymer is determined, and the activity of the enzyme is determined with the guidance of this determination.

CLM What is claimed is:
1. A method for determining the activity of a DNA polymerizing enzyme in a sample where in an initial capture step the DNA polymerizing enzyme intended for determination is captured by means of a monoclonal antibody which is immobilized on a solid carrier and is capable of binding said DNA polymerizing enzyme without detrimentally affecting the DNA polymerizing enzyme activity, in a subsequent step contaminants and disturbing factors are removed, nucleotide polymerization for the formation of DNA is started by the addition of a reaction solution containing a primer, a template and a nucleotide substrate, comprising choosing reaction conditions for the polymerizing reaction to result in permanent association between the newly synthesized DNA polymer and the captured DNA polymerizing enzyme, optionally, washing away nucleotide substrate, primer, template and reaction solution from the DNA polymer obtained, determining the amount of nucleotide incorporated in said DNA polymer associated with the immobilized DNA polymerizing enzyme and through this determination of the amount of nucleotide determining the DNA polymerizing enzyme activity.

2. A method according to claim 1, wherein the enzyme intended for the determination is DNA polymerase.

3. A method according to claim 1 wherein the DNA polymerizing enzyme is reverse transcriptase.

4. A method according to claim 1, comprising the determination of incorporated nucleotide by radioactive marking of the nucleotide.

5. A method according to claim 2, comprising the determination of incorporated nucleotide by radioactive marking of the nucleotide.

6. A method according to claim 3, comprising the determination of

incorporated nucleotide by radioactive marking of the nucleotide.

7. A method according to claim 1, comprising the determination of incorporated nucleotide by non-radioactive marking of the nucleotide.

8. A method according to claim 2, comprising the determination of incorporated nucleotide by non-radioactive marking of the nucleotide.

9. A method according to claim 3, comprising the determination of incorporated nucleotide by non-radioactive marking of the nucleotide.

10. A method according to claim 1, comprising the determination of incorporated nucleotide by colorimetric assay.

11. The method according to claim 1, for the determination of HIV1 RT activity in a sample.

12. The method according to claim 1, for the determination of Herpes Simplex DNA polymerase activity in a sample.

L12 ANSWER 21 OF 35 USPATFULL
1999:128346 Polymerase chain reaction assays for monitoring antiviral therapy and making therapeutic decisions in the treatment of acquired immunodeficiency syndrome.
Merigan, Thomas C., Portola Valley, CA, United States
Katzenstein, David A., Menlo Park, CA, United States
Holodniy, Mark, Mountain View, CA, United States
Leland Stanford Junior University, Palo Alto, CA, United States (U.S. corporation)
US 5968730 19991019
APPLICATION: US 1995-470885 19950606 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods of monitoring, via polymerase chain reaction, the clinical progression of ***human*** ***immunodeficiency*** ***virus*** infection and its response to antiretroviral therapy. According to the invention, polymerase chain reaction assays may be used to predict immunological decline and to identify, at an early stage, patients whose infection has become resistant to a particular antiretroviral drug regimen.

CLM What is claimed is:
1. A method of evaluating the effectiveness of anti- ***HIV*** therapy of a patient comprising: (i) collecting a plasma sample from an ***HIV*** -infected patient who is being treated with an antiretroviral agent; (ii) amplifying the ***HIV*** -encoding nucleic acid in the plasma sample using ***HIV*** primers in about 30 cycles of PCR; and (iii) testing for the presence of ***HIV*** -encoding nucleic acid, in the product of the PCR; in which the absence of detectable ***HIV*** -encoding nucleic acid correlates positively with the conclusion that the antiretroviral agent is therapeutically effective.

2. The method of claim 1 in which the ***HIV*** primers are SK38 and SK39.

3. The method of claim 1 in which one ***HIV*** primer is primer NE1 (5'-TCATTGACAGTCCAGCT-3') (SEQ ID NO:2).

4. The method of claim 1 in which one ***HIV*** primer is primer A (5'-TTCCCATTAGTCCTATT-3') (SEQ ID NO:1).

5. The method of claim 1, 2, 3 or 4 in which the antiretroviral agent is zidovudine.

6. A method of evaluating the effectiveness of anti- ***HIV*** therapy of a patient comprising: (i) collecting a plasma sample from an ***HIV*** -infected patient who is being treated with an antiretroviral agent; (ii) amplifying the ***HIV*** -encoding nucleic acid in the plasma sample using ***HIV*** primers in about 30 cycles of PCR; and (iii) testing for the presence of ***HIV*** -encoding nucleic acid in the product of the PCR; in which the presence of detectable ***HIV*** -encoding nucleic acid correlates positively with the conclusion that the antiretroviral agent is therapeutically ineffective.

7. A method of evaluating the effectiveness of anti- ***HIV*** therapy of a patient comprising: (i) collecting a plasma sample from an ***HIV*** -infected patient who is being treated with an antiretroviral agent; (ii) amplifying the ***HIV*** -encoding nucleic acid in the plasma sample using ***HIV*** primers in about 30 cycles of PCR; and (iii) testing for the presence of ***HIV*** -encoding nucleic acid in the product of the PCR; in which the presence of detectable ***HIV*** -encoding nucleic acid correlates positively with an absolute CD4 count of less than 200 cells per cubic millimeter.

8. A method of evaluating the effectiveness of anti- ***HIV*** therapy of a patient comprising: (i) collecting a plasma sample from an ***HIV*** -infected patient who is being treated with an antiretroviral agent; (ii) amplifying the ***HIV*** -encoding nucleic acid in the plasma sample using ***HIV*** primers in about 30 cycles of PCR; and (iii) testing for the presence of ***HIV*** -encoding nucleic acid sequence in the product of the PCR; in which the absence of detectable ***HIV*** -encoding nucleic acid correlates positively with an absolute CD4 count of greater than 200 cells per cubic millimeter.

9. A method of evaluating the effectiveness of anti- ***HIV*** therapy of a patient comprising (i) collecting a plasma sample from an ***HIV*** -infected patient who is being treated with an antiretroviral agent; (ii) amplifying the ***HIV*** -encoding nucleic acid in the plasma sample using ***HIV*** primers in about 30 cycles of PCR; and (iii) measuring the ***HIV*** RNA copy number using the product of the PCR, in which an ***HIV*** RNA copy number greater than about 500 per 200 ul of plasma correlates positively with the conclusion that the antiretroviral agent is therapeutically ineffective.

10. The method of claim 9 in which the ***HIV*** primers are SK38 and SK39.

11. The method of claim 9 in which one ***HIV*** primer is primer NE1 (5'-TCATTGACAGTCCAGCT-3') (SEQ ID NO:2).

12. The method of claim 9 in which one ***HIV*** primer is primer A (5'-TTCCCATTAGTCCTATT-3') (SEQ ID NO:1).

13. The method of claim 9, 10, 11 or 12 in which the antiretroviral agent is zidovudine.

14. A method of evaluating the effectiveness of anti- ***HIV*** therapy of a patient comprising: (i) collecting a plasma sample from an ***HIV*** -infected patient who is being treated with an antiretroviral agent; (ii) amplifying the ***HIV*** -encoding nucleic acid in the plasma sample using ***HIV*** primers in about 30 cycles of PCR; and

(iii) measuring the ***HIV*** RNA copy number using the product of the PCR, in which an ***HIV*** RNA copy number less than about 200 per 200 ul of plasma correlates positively with the conclusion that the anti- ***HIV*** agent is therapeutically effective.

15. The method of claim 14 in which the ***HIV*** primers are SK38 and SK39.

16. The method of claim 14 in which one ***HIV*** primer is primer NE1 (5'-TCATTGACAGTCCAGCT-3') (SEQ ID NO:2).

17. The method of claim 14 in which one ***HIV*** primer is primer A (5'-TTCCCATTAGTCCTATT-3') (SEQ ID NO:1).

18. The method of claim 14, 15, 16 or 17 in which the antiretroviral agent is zidovudine.

19. A method of evaluating the effectiveness of anti- ***HIV*** therapy of a patient comprising (i) collecting one pre-treatment plasma sample from an ***HIV*** -infected patient who is about to be treated with an antiretroviral agent; (ii) collecting a post-treatment plasma sample from the ***HIV*** -infected patient after the patient has been treated with the antiretroviral agent; (iii) amplifying the ***HIV*** -encoding nucleic acid in the pre-treatment and post-treatment plasma samples using ***HIV*** primers in about 30 cycles of PCR; (iv) measuring the ***HIV*** RNA copy number using the products of the PCRs of step (iii); and (v) comparing the ***HIV*** RNA copy number in pre-treatment and post-treatment plasma samples, in which a ratio of ***HIV*** RNA copy number in pre-treatment and post-treatment plasma samples of greater than about 4 to 1 correlates positively with the conclusion that the anti- ***HIV*** agent is therapeutically effective.

20. The method of claim 19 in which the ***HIV*** primers are SK38 and SK39.

21. The method of claim 19 in which one ***HIV*** primer is primer NE1 (5'-TCATTGACAGTCCAGCT-3') (SEQ ID NO:2).

22. The method of claim 19 in which one ***HIV*** primer is primer A (5'-TTCCCATTAGTCCTATT-3') (SEQ ID NO:1).

23. The method of claim 19, 20, 21 or 22 in which the antiretroviral agent is zidovudine.

L12 ANSWER 13 OF 35 USPATFULL
2000:61379 In vitro method for predicting the evolutionary response of ***HIV*** protease to a drug targeted thereagainst.
Melnick, Laurence M., Watertown, MA, United States
Heefner, Donald L., Hudson, MA, United States
Sepracor, Inc., Marlborough, MA, United States (U.S. corporation)
US 6063562 20000516
APPLICATION: US 1995-420003 19950410 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An in vitro method for predicting the identity of distinct, first-generation, ***drug*** - ***resistant*** , biologically-active, ***HIV*** protease ***mutants*** that may emerge in vivo in response to a drug targeted thereagainst. In a preferred embodiment, the in vitro method comprises the steps of (a)

preparing, in the presence of the drug, a comprehensive library of all first-generation ***mutants*** of the protease differing therefrom by at least one and preferably no more than three amino acid substitutions, each of the protease ***mutants*** being generated as part of a polyprotein with the ***HIV*** ***reverse*** ***transcriptase*** protein; (b) isolating, in vitro, first-generation, ***drug*** - ***resistant*** , biologically-active, ***mutant*** proteases from said library by assaying for biological activity of the ***reverse*** ***transcriptase*** protein; and (c) identifying the distinct, first-generation, biologically-active, ***mutant*** proteases so isolated. The present invention also relates to an in vitro method for evaluating the efficacy of a drug against a biologically-active ***mutant*** or wild-type form of ***HIV*** protease, said method comprising the steps of (a) providing a ***mutant*** polyprotein, said ***mutant*** polyprotein including a biologically-inactive ***mutant*** form of the protease linked to ***HIV*** ***reverse*** ***transcriptase*** by one or more sites cleavable by the biologically-active or wild-type form of the protease; (b) adding the drug to the ***mutant*** polyprotein; (c) then, adding the biologically-active or wild-type form of the protease to the ***mutant*** polyprotein; and (d) then, assaying for the presence of biological activity for ***reverse*** ***transcriptase*** , whereby the presence of ***reverse*** ***transcriptase*** activity indicates that the drug is not efficacious against the biologically-active ***mutant*** or wild-type form of the protease tested. The present invention further relates to a kit for evaluating the efficacy of a drug against a biologically-active ***mutant*** or wild-type form of ***HIV*** protease.

CLM What is claimed is:

1. A kit for evaluating, in vitro, the efficacy of a drug against a biologically-active ***mutant*** or wild-type form of a first protein, the first protein being a protease that is natively expressed as part of a polyprotein with a second protein, the second protein having a biological activity which is catalyzed by cleavage of the polyprotein by the protease, said kit comprising: (a) a ***mutant*** polyprotein, said ***mutant*** polyprotein including the second protein, a biologically-inactive ***mutant*** form of the protease, and one or more sites cleavable by the biologically-active or wild-type form of the protease in such a way as to activate the second protein; (b) a biologically-active ***mutant*** or wild-type form of the protease which, when combined with the ***mutant*** polyprotein in the absence of an effective drug thereagainst, cleaves the ***mutant*** polyprotein in such a way as to activate the second protein; and (c) means for detecting the presence of biological activity for the second protein.

2. The kit of claim 1 wherein the ***mutant*** polyprotein has the same number and type of cleavage sites needed to cleave the second protein from the ***mutant*** polyprotein as are found in the naturally-occurring polyprotein.

3. The kit of claim 1 wherein the protease is ***HIV*** protease.

4. The kit of claim 3 wherein the second protein is ***reverse*** ***transcriptase*** .

5. An in vitro protease ***assay*** kit for a protease of the type that is natively expressed as part of a polyprotein with a second protein, the second protein having a biological activity which is

catalyzed by cleavage of the polyprotein by the protease, said ***assay*** kit comprising: (a) a ***mutant*** polyprotein, said ***mutant*** polyprotein including a biologically-inactive ***mutant*** form of the protease, said second protein, and one or more sites cleavable by an active form of the protease in such a way as to activate the second protein; and (b) means for detecting the presence of biological activity for the second protein.

6. The in vitro protease ***assay*** kit of claim 5 wherein the ***mutant*** polyprotein has the same number and type of cleavage sites needed to cleave the second protein from the ***mutant*** polyprotein as are found in the naturally-occurring polyprotein.

7. The in vitro protease ***assay*** kit of claim 5 wherein the protease is ***HIV*** protease.

8. The in vitro protease ***assay*** kit of claim 7 wherein the second protein is ***reverse*** ***transcriptase*** .

9. An in vitro method for evaluating the efficacy of a drug against a biologically active protease chosen from a wild-type protease and a ***mutant*** form of a protease, the protease being natively expressed as part of a polyprotein that includes a second protein whose biological activity is dependent upon cleavage of the polyprotein by the protease, said method comprising the steps of: (a) providing a proteolytically incompetent ***mutant*** of the polyprotein that includes (i) a biologically inactive ***mutant*** of the protease, (ii) the second protein, and (iii) at least one site cleavable by a biologically active form of the protease to activate the second protein; (b) adding a drug to be evaluated to the proteolytically incompetent ***mutant*** of the polyprotein; (c) adding the biologically active form of the protease to the product of step (b); and (d) assaying the product of step (c) for the presence of biological activity for the second protein, wherein the absence of biological activity for the second protein indicates that the drug has efficacy against the biologically active form of the protease.

10. The method of claim 9 wherein the proteolytically incompetent ***mutant*** of the polyprotein has the same number and type of cleavage sites needed for cleavage by the biologically active form of the protease as are found in the polyprotein.

11. The method of claim 9 wherein the protease is ***HIV*** protease.

12. The method of claim 11 wherein the second protein is ***reverse*** ***transcriptase*** .

13. The method of claim 9 wherein the biologically active protease is a biologically active ***mutant*** and said protease is ***drug*** - ***resistant*** to a second drug, said second drug being a protease inhibitor.

14. The method of claim 9 wherein the biologically active protease is a biologically active ***mutant*** and said ***mutant*** protease has been identified in a pathogen isolated from a human.

L13 ANSWER 1 OF 10 MEDLINE
2001379388 Document Number: 21329505. PubMed ID: 11435603. Correlation between viral resistance to zidovudine and resistance at the reverse transcriptase level for a panel of human immunodeficiency virus type 1 mutants. ***Lennerstrand J*** ; Hertogs K; Stammers D K; Larder B A. (Virco UK, Ltd., Cambridge CB4 0GA, United Kingdom.. johan.lennerstrand@impi.ki.se) . JOURNAL OF VIROLOGY, (2001 Aug) 75 (15) 7202-5. Journal code: KCV; 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Using a large panel of human immunodeficiency virus type 1 site-directed mutants, we have observed a higher correlation than has previously been demonstrated between zidovudine (AZT)-triphosphate resistance data at the reverse transcriptase (RT) level and corresponding viral AZT resistance. This enhanced-resistance effect at the RT level was seen with ATP and to a lesser extent with PP(i) when ATP was added at physiological concentrations. The ATP-dependent mechanism (analogous to pyrophosphorolysis) appears to be dominant in the mutants bearing the D67N and K70R or 69 insertion mutations, whereas the Q151M mutation seems independent of ATP for decreased binding to AZT-triphosphate.

L13 ANSWER 2 OF 10 MEDLINE
2001345052 Document Number: 21301176. PubMed ID: 11408240. Biochemical mechanism of human immunodeficiency virus type 1 reverse transcriptase resistance to stavudine. ***Lennerstrand J*** ; Stammers D K; Larder B A. (Virco UK, Ltd, Cambridge CB4 0GA, United Kingdom.. johan.lennerstrand@impi.ki.se) . ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, (2001 Jul) 45 (7) 2144-6. Journal code: 6HK; 0315061. ISSN: 0066-4804. Pub. country: United States. Language: English.

AB We have found a close correlation between viral stavudine (d4T) resistance and resistance to d4T-triphosphate at the human immunodeficiency virus type 1 reverse transcriptase (RT) level. RT from site-directed mutants with 69S-XX codon insertions and/or conventional zidovudine resistance mutations seems to be involved in an ATP-dependent resistance mechanism analogous to pyrophosphorolysis, whereas the mechanism for RT with the Q151M or V75T mutation appears to be independent of added ATP for reducing binding to d4T-triphosphate.

L13 ANSWER 10 OF 10 MEDLINE
91273820 Document Number: 91273820. PubMed ID: 1711325. Carrier bound templates for single tube reverse transcriptase assays and for combined purification and activity analyses, with special reference to HIV. Gronowitz J S; Neumuller M; ***Lennerstrand J*** ; Bhikhabhai R; Unge T; Weltman H; Kallander C F. (Research Unit for Replication Enzymology, Uppsala University, Sweden.) BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, (1991 Feb) 13 (1) 127-42. Journal code: AHF; 8609465. ISSN: 0885-4513. Pub. country: United States. Language: English.

AB Polyriboadenosine (prA) was coupled to polycarbonate macrobeads or magnetic beads. The efficiency of the beads and of prA-Sepharose, after priming with odT, as templates in activity assays of purified AMV- and HIV-reverse transcriptase (RT), using [125I]iododeoxyuridine-triphosphate as substrate, was studied. Although the use of immobilized templates, compared with soluble template, resulted in a decreased total molar turnover, it did not affect the sensitivity of the assay for detecting RT. The utility of the new assay was analyzed by mixing purified AMV- or HIV-Rt with different dilutions of the untreated clinical specimen. This showed that RT activity was unaffected by 100 microliters of an extract of whole blood cells resuspended to their original blood volume and diluted

1/64, and also by 100 microliters of serum diluted 1/64. To improve the utility of the assay at the inhibitory concentrations of clinical specimens, the following procedure was adopted: the sample to be analyzed was incubated with the carrier bound template in order to allow the RT to bind, the carrier was washed to remove inhibitory factors, and the reaction components were then added to determine the amount of bound RT. This procedure greatly enhanced the recovery of RT activity from crude specimens and made the direct detection of HIV-RT possible. The assay is easily automated and useful for RT determination in multiple samples and for determining RT-inhibiting substances such as substrate analogs and antibodies.

L19 ANSWER 3 OF 3 MEDLINE
1999140284 Document Number: 99140284. PubMed ID: 10048915. Closing in on ***HIV*** drug resistance. ***Larder B A*** ; Stammers D K. NATURE STRUCTURAL BIOLOGY, ***(1999 Feb)*** 6 (2) 103-6. Journal code: B98; 9421566. ISSN: 1072-8368. Pub. country: United States. Language: English.

L19 ANSWER 1 OF 3 MEDLINE
1999359258 Document Number: 99359258. PubMed ID: 10428920. A family of insertion mutations between codons 67 and 70 of ***human*** ***immunodeficiency*** ***virus*** type 1 ***reverse*** ***transcriptase*** confer multinucleoside analog resistance. ***Larder B A*** ; Bloor S; Kemp S D; Hertogs K; Desmet R L; Miller V; Sturmer M; Staszewski S; Ren J; Stammers D K; Stuart D I; Pauwels R. (Virco UK, Ltd., Cambridge, CB4 4GH, United Kingdom.. brendan.larder@virco.co.uk) . ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, ***(1999 Aug)*** 43 (8) 1961-7. Journal code: 6HK; 0315061. ISSN: 0066-4804. Pub. country: United States. Language: English.

AB To investigate the occurrence of multinucleoside analog resistance during therapy failure, we surveyed the drug susceptibilities and genotypes of nearly 900 ***human*** ***immunodeficiency*** ***virus*** type 1 (***HIV*** -1) samples. For 302 of these, the 50% inhibitory concentrations of at least four of the approved nucleoside analogs had fourfold-or-greater increases. Genotypic analysis of the ***reverse*** ***transcriptase*** (***RT***)-coding regions from these samples revealed complex mutational patterns, including the previously recognized codon 151 multidrug resistance cluster. Surprisingly, high-level multinucleoside resistance was associated with a diverse family of amino acid insertions in addition to "conventional" point mutations. These insertions were found between ***RT*** codons 67 and 70 and were commonly 69Ser-(Ser-Ser) or 69Ser-(Ser-Gly). Treatment history information showed that a common factor for the development of these variants was AZT (3'-azido-3'-deoxythymidine, zidovudine) therapy in combination with 2',3'-dideoxyinosine or 2',3'-dideoxycytidine, although treatment patterns varied considerably. Site-directed mutagenesis studies confirmed that 69Ser-(Ser-Ser) in an AZT resistance mutational background conferred simultaneous resistance to multiple nucleoside analogs. The insertions are located in the "fingers" domain of ***RT*** . Modelling the 69Ser-(Ser-Ser) insertion into the ***RT*** structure demonstrated the profound direct effect that this change is likely to have in the nucleoside triphosphate binding site of the ***enzyme*** . Our data highlight the increasing problem of ***HIV*** -1 multidrug resistance and underline the importance of continued resistance surveillance with appropriate, sufficiently versatile genotyping technology and phenotypic drug susceptibility analysis.

L25 ANSWER 4 OF 4 MEDLINE
91324391 Document Number: 91324391. PubMed ID: 1713913. A sensitive non-isotopic assay specific for HIV-1 associated ***reverse***

transcriptase . Porstmann T; Meissner K; Glaser R; Dopel S H; Sydow G. (Department of Medical Immunology, Medical School (Charite), Humboldt University Berlin, F.R.G.) JOURNAL OF VIROLOGICAL METHODS, (1991 Feb-Mar) 31 (2-3) 181-8. Journal code: HQR; 8005839. ISSN: 0166-0934. Pub. country: Netherlands. Language: English.

AB A sensitive non-isotopic assay for specific detection of ***reverse*** ***transcriptase*** (***RT***) of the human immunodeficiency virus type 1 (HIV-1) is described using 5-bromo-2'-deoxyuridine triphosphate (***BrdUTP***) instead of tritiated thymidine triphosphate. After the ***RT*** reaction the template primer is degraded by alkaline hydrolysis. Single-stranded poly.(BrdU) is detected in an immunoenzymometric assay using monoclonal anti-BrdU antibodies. The specificity of the assay is demonstrated by the isolation of ***RT*** from virus lysate by an insolubilised monoclonal anti-HIV-1 ***RT*** antibody prior to the ***RT*** reaction. Immunological ***RT*** binding leads to a tenfold increase in analytical sensitivity since substances inhibiting the ***RT*** reaction can be removed. This non-isotopic assay is some 30 times more sensitive than the classical radioisotopic ***RT*** assay. In terms of ***RT*** determination, however, there is a good correlation between these tests (r = 0.96). Several filtrations are no longer necessary to remove non-incorporated nucleotides. The test can be adapted to microtitre plates and hence is easy to automate.

L25 ANSWER 3 OF 4 MEDLINE

96206778 Document Number: 96206778. PubMed ID: 8639277. A sensitive assay for the quantification of ***reverse*** ***transcriptase*** activity based on the use of carrier-bound template and non-radioactive-product detection, with special reference to human-immunodeficiency-virus isolation. Ekstrand D H; Awad R J; Kallander C F; Gronowitz J S. (Department of Medical Genetics, Uppsala University, Sweden.) BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, (1996 Apr) 23 (Pt 2) 95-105. Journal code: AHF; 8609465. ISSN: 0885-4513. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A non-radioactive 96-well microtitre plate ***reverse*** ***transcriptase*** (***RT***) assay, based on the use of covalently bound riboadenosine homopolymer in the wells and 5-bromodeoxyuridined 5'-triphosphate (***BrdUTP***) as dNTP, is described. The whole assay is performed in a single well, including the quantitative detection of incorporated BrdU, which is performed immunologically using alkaline phosphatase-conjugated anti-BrdU antibody and colorometric reading. The system also allows the use of variable amounts of primer. The kinetics and characteristics of the assay using ***BrdUTP*** is similar to the use of [3H]dTTP. The sensitivity of the assay can be varied either by altering the duration of ***RT*** assay time and/or by prolonging the alkaline phosphatase reaction. Thus the assay can detect < 0.02 pg of recombinant human-immunodeficiency-virus (HIV) type I ***RT*** , < 0.005 m unit of avian-myeloblastosis-virus ***RT*** or < 0.02 m unit of recombinant Moloney-murine-leukaemia-virus ***RT*** . The assay was found to be useful with various types of cell-culture material, and a comparative study of 16 HIV-infected lymphocyte cultures, using 10 microliters of supernatant medium for ***RT*** assay and 22.5 microliters for p24 antigen assay showed that the new ***RT*** assay was at least 25-fold more sensitive than the p24 antigen assay. The results also show a good correlation between the ***RT*** activities found and the p24-antigen level detected, with exception for HIV2 isolates, as they only became positive in the ***RT*** assay. The technical performance and the capacity of the test compared with other available ***RT*** kits is discussed, as well as its use for other applications.

L25 ANSWER 2 OF 4 MEDLINE
97405876 Document Number: 97405876. PubMed ID: 9260920. Immunoseparation and immunodetection of nucleic acids labeled with halogenated nucleotides. Haider S R; Juan G; Traganos F; Darzynkiewicz Z. (The Cancer Research Institute, New York Medical College, Valhalla 10595, USA.. raza_haider@nymc.edu) . EXPERIMENTAL CELL RESEARCH, (1997 Aug 1) 234 (2) 498-506. Journal code: EPB; 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB A novel methodology for labeling, isolation, and detection of nucleic acids is described. Nucleic acid isolation is based on in vivo or in vitro incorporation of BrU or BrdU to either RNA or DNA, respectively, followed by immunoprecipitation of the labeled nucleic acid utilizing anti-BrdU MoAb, which crossreacts with BrU, attached to solid particles. Filter-bound bromine-labeled DNA or RNA was detected by immunoblotting with anti-BrdU MoAb, by a combined Southern/Western or Northern/Western approach, respectively. This method was applied to isolate and detect rRNA and mRNA from human cells, plasmid DNA from bacterial cells, and in vitro synthesized DNA. Newly transcribed BrU-labeled mRNA was recovered from the immunoprecipitates and analyzed by ***RT*** -PCR to study phorbol ester-mediated regulation of interleukin 1 gene transcription in human leukemic HL-60 or lymphoma U937 cells. The plasmid DNAs were isolated by immunoprecipitation from transformed bacterial cultures that were grown in the presence of BrdU and were detected immunochemically on filters. Likewise, the products of ***RT*** -PCR and Klenow polymerase-catalyzed DNA synthesis in which dTTP was replaced with ***BrdUTP*** were detected by immunoblotting. Since the method allows one to selectively separate or detect nucleic acids only synthesized during a pulse of the precursor, it can uniquely be used to identify nascent gene transcripts or the transcripts synthesized within specific time windows, e.g., after induction of differentiation, carcinogenesis, or drug treatment, and distinguish such transcripts from preexisting ones. In addition, this approach offers a simple and inexpensive alternative for preparing labeled DNA as well as RNA probes for use in a variety of hybridization protocols. Due to the low toxicity of BrU and BrdU, this approach can be used in analysis of gene transcription or DNA replication in vivo.

L25 ANSWER 1 OF 4 MEDLINE
1999268838 Document Number: 99268838. PubMed ID: 10334955. Colorimetric capture assay for human-immunodeficiency-virus-I ***reverse*** ***transcriptase*** activity. Rytting A S; Akerblom L; Gronowitz J S; Kallander C F. (Department of Genetics and Pathology, Section of Medical Genetics, Uppsala University, BMC, Box 578, SE-751 23 Uppsala, Sweden.) BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, (1999 Jun) 29 (Pt 3) 241-50. Journal code: AHF; 8609465. ISSN: 0885-4513. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The development of a colorimetric capture assay for HIV-1 ***reverse*** ***transcriptase*** (***RT***) activity is described. This assay consisted of three basic steps: enzyme purification, ***RT*** reaction and product detection, which were all performed in the same microtitre plate. Mouse monoclonal anti- ***RT*** antibodies of subclass G2a were bound by polyclonal goat anti-(mouse IgG2a) immobilized in the wells of a microtitre plate. The monoclonal antibodies (mAbs) were selected for their ability to bind HIV-1 ***RT*** without hampering the polymerase activity. The assay system first involved the ***RT*** 's adherence to the immobilized mAbs. Non-specific enzymes and other impurities were removed by a simple wash, after which an ***RT*** reaction mixture containing ***BrdUTP*** as nucleotide substrate was added. After the ***RT*** reaction substrate and product had been separated by washing of

the plate, the amount of BrdUMP-DNA in the wells was finally detected with alkaline-phosphatase-conjugated mouse anti-BrdU antibodies of subclass IgG1. The background signal in this system was similar to the signals obtained with control wells coated with BSA only. A detection limit of 1.2 micro-units of ***RT*** activity, corresponding to 0.3 pg of ***RT*** protein, was obtained for the capture assay when applying colorimetric product detection. The assay detected RTs from HIV-1 subtypes A and B and one of the two D type isolates tested. None of the five non-HIV-1 RTs tested was found positive. At least 50 microl of human serum or plasma per sample could be included in the capture assay without adverse effects on the recovery of the ***RT*** activity.

L30 ANSWER 6 OF 8 MEDLINE
96424751 Document Number: 96424751. PubMed ID: 8827212. The role of ***HIV*** type 1 phenotype and genotype in long-term responders to zidovudine therapy. Angarano G; Monno L; Vivirito M C; Appice A; Romanelli C; Giannelli A; La Grasta L; Fracasso C; Fiore J R; Milazzo F. (Clinic of Infectious Diseases, University of Bari, Italy.) AIDS RESEARCH AND HUMAN RETROVIRUSES, (1996 Jul 20) 12 (11) 969-75. Journal code: ART; 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB We performed a cross-sectional and partly retrospective virological evaluation of 31 long-term responders (LTRs) to zidovudine (ZDV) (persistent increase in the CD4+ cell counts without progression of ***HIV*** infection throughout a period of ZDV therapy > 3 years) and 17 well-matched controls who developed a marked immunological deterioration over a 24-month period of ZDV therapy. The biological phenotype of ***HIV*** -1 was assessed by testing the capacity of the isolates to replicate in the MT-2, HUT-78, C-8166, and U-937 T cell lines, and mutations at codons 215 and 41 of ***RT*** were checked in proviral DNA from uncultured PBMCs. Show/low non-syncytium-inducing (S/L-NSI) and rapid/high syncytium-inducing (R/H-SI) variants were detected in 25 (81%) and 2 (6%) LTRs, respectively. ***HIV*** -1 could not be isolated in the remaining four LTRs (13%). Conversely, 12 of 17 (71%) controls yielded R/H-SI variants. Conversion from the S/L-NSI to R/H to R/H-SI phenotype occurred in 5 controls but in none of the 18 LTRs tested. Mutant sequences in proviral DNA from control PBMCs were consistently detected (94%), while a wild-type sequence of the residues investigated was found in the majority of LTRs (77%). In our series, patients who received immunological and clinical benefits even after prolonged ZDV treatment had S/L-NSI viruses and a low risk to develop ZDV resistance. Conversely, subjects who demonstrated an immunological and clinical deterioration yielded R/H-SI variants or shifted from S/L-NSI to R/H-SI phenotypes and were at higher risk to develop mutations indicating ZDV resistance.

L32 ANSWER 3 OF 4 MEDLINE
2000033659 Document Number: 20033659. PubMed ID: 10565938. Mutation patterns of the ***reverse*** ***transcriptase*** and protease genes in ***human*** ***immunodeficiency*** ***virus*** type 1-infected patients undergoing combination therapy: survey of 787 sequences. Yahi N; Tamalet C; Tourres C; Tivoli N; Ariasi F; Volot F; Gastaut J A; Gallais H; Moreau J; Fantini J. (Laboratoire de Virologie, UF SIDA, CHRU de la Timone, 13005 Marseille, France.) JOURNAL OF CLINICAL MICROBIOLOGY, (1999 Dec) 37 (12) 4099-106. Journal code: HSH; 7505564. ISSN: 0095-1137. Pub. country: United States. Language: English.

AB The aim of the present study was to evaluate the resistance-associated mutations in 302 ***human*** ***immunodeficiency*** ***virus*** type 1 (***HIV*** -1)-infected patients receiving combination therapy and monitored in Marseille, France, hospitals from January 1997 to June 1998. In the ***reverse*** ***transcriptase*** (***RT***)

gene, the most frequent mutations were found at codons 215 (53%), 41 (34%), and ***67*** , ***70*** , 184, and 210 (>20%). One deletion and two insertions in the beta3-beta4 hairpin loop of the finger subdomain (codon ***69***) were detected. Interesting associations and/or exclusions of specific mutations were observed. In 96% of ***RT*** genes, a mutation at codon ***70*** (most frequently, K70R) was associated with a wild-type genotype at position 210 (P < 10(-5)). Similarly, a mutation at codon 210 (most frequently, L210W) was generally associated with mutations at codons 41 (92%) and 215 (96%) but not at codon 219 (16%) or codon ***70*** (4%) (P < 10(-5)). In the protease gene, the most prevalent mutations were at codons 63 (84%), followed by codons 10, 36, 71, 77, and 93 (ca. 20%). As for ***RT*** , pairwise associations of mutations were observed. Analysis of the mutation patterns for patients with undetectable ***HIV*** -1 loads revealed a high proportion (65%) of wild-type ***RT*** genotypes but only 18% wild-type protease genotypes. For patients with high viral loads (>100,000 copies/ml), more than 50% of the ***RT*** and protease genes displayed three or more mutations. The significant correlation between the level of viremia in plasma and the number of resistance mutations in the protease (P = 0.007) and ***RT*** (P = 0.00078) genes strengthens the importance of defining the genotype of the predominant ***HIV*** -1 quasispecies before initiating antiretroviral therapy.

L32 ANSWER 4 OF 4 MEDLINE
95194708 Document Number: 95194708. PubMed ID: 7534096. Natural occurrence of ***drug*** ***resistance*** mutations in the ***reverse*** ***transcriptase*** of ***human*** ***immunodeficiency*** ***virus*** type 1 isolates. Najera I; Richman D D; Olivares I; Rojas J M; Peinado M A; Perucho M; Najera R; Lopez-Galindez C. (Centro Nacional de Biologia Celular y Retrovirus, Instituto de Salud Carlos III, Madrid, Spain.) AIDS RESEARCH AND HUMAN RETROVIRUSES, (1994 Nov) 10 (11) 1479-88. Journal code: ART; 8709376. ISSN: 0889-2229. Pub. country: United States. Language: English.

AB ***Reverse*** ***transcriptase*** -associated amino acid substitutions related to ddC, d4T, and nevirapine resistance have been found in isolates of ***human*** ***immunodeficiency*** ***virus*** type 1 (***HIV*** -1) from patients treated with AZT only. Sequence analysis of 23 isolates documented the presence of 4 unexpected mutations at amino acid residues related to ***drug*** ***resistance*** . Two isolates contained an aspartic residue in codon ***69*** associated with ddC resistance, and another a change in codon 75 associated with resistance to d4T. The Y-to-C alteration in codon 181 associated with nevirapine resistance was observed in another isolate after serial passage in cell culture in the absence of drug. Changes in substitution patterns were also noted after serial passage of four AZT resistant isolates in cell culture without inhibitors. One of the strains showed changes in codons ***67*** and ***70*** to wild-type residues. Clonal analysis showed that this alteration occurred by the selection during cell culture passage of the wild-type genotype, which was present as a minority subpopulation in the initially resistant virus stock, rather than to genetic ***reversion*** . In summary, we present evidence documenting the presence of mutations associated with ***drug*** ***resistance*** in the absence of drug treatment and supporting the role played by gentic variability in the emergence of ***HIV*** -1 antiviral resistance.

L34 ANSWER 1 OF 3 MEDLINE
2001379388 Document Number: 21329505. PubMed ID: 11435603. Correlation between viral resistance to zidovudine and resistance at the ***reverse*** ***transcriptase*** level for a panel of ***human***

immunodeficiency ***virus*** type 1 mutants. Lennerstrand J; Hertogs K; Stammers D K; Larder B A. (Virco UK, Ltd., Cambridge CB4 0GA, United Kingdom.. johan.lennerstrand@impi.ki.se) . JOURNAL OF VIROLOGY, (2001 Aug) 75 (15) 7202-5. Journal code: KCV; 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Using a large panel of ***human*** ***immunodeficiency*** ***virus*** type 1 site-directed mutants, we have observed a higher correlation than has previously been demonstrated between zidovudine (AZT)-triphosphate resistance data at the ***reverse*** ***transcriptase*** (***RT***) level and corresponding viral AZT resistance. This enhanced-resistance effect at the ***RT*** level was seen with ATP and to a lesser extent with PP(i) when ATP was added at physiological concentrations. The ATP-dependent mechanism (analogous to pyrophosphorolysis) appears to be dominant in the mutants bearing the D67N and K70R or ***69*** ***insertion*** ***mutations*** , whereas the Q151M mutation seems independent of ATP for decreased binding to AZT-triphosphate.

L34 ANSWER 2 OF 3 MEDLINE
1999359258 Document Number: 99359258. PubMed ID: 10428920. A family of ***insertion*** ***mutations*** between codons ***67*** and ***70*** of ***human*** ***immunodeficiency*** ***virus*** type 1 ***reverse*** ***transcriptase*** confer multinucleoside analog resistance. Larder B A; Bloor S; Kemp S D; Hertogs K; Desmet R L; Miller V; Sturmer M; Staszewski S; Ren J; Stammers D K; Stuart D I; Pauwels R. (Virco UK, Ltd., Cambridge, CB4 4GH, United Kingdom.. brendan.larder@virco.co.uk) . ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, (1999 Aug) 43 (8) 1961-7. Journal code: 6HK; 0315061. ISSN: 0066-4804. Pub. country: United States. Language: English.

AB To investigate the occurrence of multinucleoside analog resistance during therapy failure, we surveyed the drug susceptibilities and genotypes of nearly 900 ***human*** ***immunodeficiency*** ***virus*** type 1 (***HIV*** -1) samples. For 302 of these, the 50% inhibitory concentrations of at least four of the approved nucleoside analogs had fourfold-or-greater increases. Genotypic analysis of the ***reverse*** ***transcriptase*** (***RT***)-coding regions from these samples revealed complex mutational patterns, including the previously recognized codon 151 multidrug resistance cluster. Surprisingly, high-level multinucleoside resistance was associated with a diverse family of amino acid insertions in addition to "conventional" point mutations. These insertions were found between ***RT*** codons ***67*** and ***70*** and were commonly 69Ser-(Ser-Ser) or 69Ser-(Ser-Gly). Treatment history information showed that a common factor for the development of these variants was AZT (3'-azido-3'-deoxythymidine, zidovudine) therapy in combination with 2',3'-dideoxyinosine or 2',3'-dideoxycytidine, although treatment patterns varied considerably. Site-directed mutagenesis studies confirmed that 69Ser-(Ser-Ser) in an AZT resistance mutational background conferred simultaneous resistance to multiple nucleoside analogs. The insertions are located in the "fingers" domain of ***RT*** . Modelling the 69Ser-(Ser-Ser) insertion into the ***RT*** structure demonstrated the profound direct effect that this change is likely to have in the nucleoside triphosphate binding site of the enzyme. Our data highlight the increasing problem of ***HIV*** -1 multidrug resistance and underline the importance of continued resistance surveillance with appropriate, sufficiently versatile genotyping technology and phenotypic ***drug*** ***susceptibility*** analysis.

L34 ANSWER 3 OF 3 MEDLINE
1999339711 Document Number: 99339711. PubMed ID: 10413366. Identification

of ***insertion*** ***mutations*** in ***HIV*** -1 ***reverse*** ***transcriptase*** causing multiple ***drug*** ***resistance*** to nucleoside analogue ***reverse*** ***transcriptase*** inhibitors. Sugiura W; Matsuda M; Matsuda Z; Abumi H; Okano A; Oishi T; Moriya K; Yamamoto Y; Fukutake K; Mimaya J; Ajisawa A; Taki M; Yamada K; Nagai Y. (National institute of Infectious Diseases AIDS Research Center, Tokyo, Japan.. wsugiura@nih.go.jp) . JOURNAL OF HUMAN VIROLOGY, (1999 May-Jun) 2 (3) 146-53. Journal code: DA6; 9805755. ISSN: 1090-9508. Pub. country: United States. Language: English.

AB OBJECTIVE: A novel 2-amino acid insertion between codons ***69*** and ***70*** of ***human*** ***immunodeficiency*** ***virus*** type 1 (***HIV*** -1) ***reverse*** ***transcriptase*** (***RT***) which confers multiple ***drug*** ***resistance*** has recently been reported. Independently, we have identified similar ***insertion*** ***mutations*** in Japanese hemophiliacs and attempted to analyze their emergence in conjunction with therapy regimens and their contribution to ***drug*** ***resistance*** using recombinant technology. METHODS: The plasma and peripheral blood mononuclear cells (PBMCs) of 348 ***HIV*** -1-infected hemophiliacs were screened for ***HIV*** -1 ***RT*** mutations relevant to nucleoside analogue inhibitors and isolating viruses. Contribution of each insertion to ***drug*** ***resistance*** was studied by introducing the mutations into a T-cell line-tropic NL4-3 infectious clone and testing the drug susceptibilities of the recovered virus. RESULTS: Insertion of the 2-amino acid residue was found in 4 of the 348 cases and was strongly associated with prolonged chemotherapy with zidovudine (AZT) and didanosine (ddI). The virus isolated from 1 of the 4 cases possessed the same insertion. Characterization of these virus and the recombinant NL4-3 with the insertion strongly suggested that the insertion caused resistance not only to AZT and ddI but also to lamivudine (3TC) and zalcitabine (ddC). CONCLUSION: A 2-amino acid insertion between codons ***69*** and ***70*** of ***RT*** was detected in 4 of 348 (1.1%) Japanese hemophiliacs and was found to be associated with multiple ***drug*** ***resistance*** to nucleoside analogue ***RT*** inhibitors.

L37 ANSWER 21 OF 24 MEDLINE
93054426 Document Number: 93054426. PubMed ID: 1385392. Inhibitory effects of triphosphate derivatives of oxetanocin G and related compounds on eukaryotic and viral DNA polymerases and human immunodeficiency virus ***reverse*** ***transcriptase*** . Izuta S; Shimada N; Kitagawa M; Suzuki M; Kojima K; Yoshida S. (Laboratory of Cancer Cell Biology, Nagoya University School of Medicine, Aichi.) JOURNAL OF BIOCHEMISTRY, (1992 Jul) 112 (1) 81-7. Journal code: HIF; 0376600. ISSN: 0021-924X. Pub. country: Japan. Language: English.

AB In order to clarify the biological activities of (-)-oxetanocin G, and (-)-oxetanocin A and its carbocyclic analogue, (-)-carboxetanocin G, the inhibitory effects of triphosphate derivatives of these compounds (OXT- ***GTP*** , OXT- ***ATP*** , and C-OXT- ***GTP***) on eukaryotic and viral DNA polymerases were examined. DNA polymerase alpha purified from calf thymus was weakly inhibited by OXT- ***GTP*** and OXT- ***ATP*** but strongly by C-OXT- ***GTP*** , the Ki value being 0.22 microM. On the other hand, rat DNA polymerase beta was not affected by these analogues. DNA polymerase gamma purified from bovine testes was very weakly inhibited by OXT- ***GTP*** and OXT- ***ATP*** , but not by C-OXT- ***GTP*** . DNA polymerase from herpes simplex virus type-II (HSV-II) was strongly inhibited by all three analogues, the Ki values ranging from 0.5 to 1.0 microM. Human immunodeficiency virus-encoded ***reverse*** ***transcriptase*** (HIV ***RT***) was also

strongly inhibited by these three analogues, the Ki value of C-OXT- ***GTP*** being slightly smaller than that of OXT- ***GTP*** or OXT- ***ATP*** . Analysis of products synthesized on singly primed M13 single-stranded DNA by DNA polymerase alpha, HSV-II DNA polymerase or HIV ***RT*** in the presence of the analogues revealed that OXT- ***GTP*** and C-OXT- ***GTP*** were incorporated into DNA and caused chain termination mainly at sites one or two nucleotides beyond the cytosine bases on the template.

L40 ANSWER 8 OF 14 MEDLINE
95178098 Document Number: 95178098. PubMed ID: 7532977. Fidelity of nucleic acid amplification with avian myeloblastosis virus ***reverse*** ***transcriptase*** and T7 RNA polymerase. Sooknanan R; Howes M; Read L; Malek L T. (Cangene Corporation, Mississauga, Ontario, Canada.) BIOTECHNIQUES, (1994 Dec) 17 (6) 1077-80, 1083-5. Journal code: AN3; 8306785. ISSN: 0736-6205. Pub. country: United States. Language: English.

AB The Nucleic Acid Sequence-Based Amplification (NASBA) process involves alternate steps of DNA synthesis from an RNA template and RNA synthesis from a DNA template, using avian myeloblastosis virus (AMV) ***reverse*** ***transcriptase*** and T7 RNA polymerase, respectively. The overall fidelity of the amplification process was determined by sequence analysis of cloned DNA products of NASBA reactions. An error frequency of less than 0.3% was observed in cloned DNA products from two different segments of the ***HIV*** -1 gag gene. Partial substitution of ***GTP*** with ITP in the NASBA reaction did not significantly change the fidelity of the process. An error rate of 2 x 10(-4) was calculated for the combined effects of both polymerases.

L40 ANSWER 7 OF 14 MEDLINE
97035725 Document Number: 97035725. PubMed ID: 8881373. Purine ribonucleotide content in infected ***HIV*** - ***RT*** + and ***HIV*** - ***RT*** - lymphoblastoid cell lines. Carlucci F; Tabucchi A; Rosi F; Pagani R; Leoncini R; Pizzichini M; Marinello E. (Institute of Biochemistry and Enzymology, University of Siena, Italy.) BIOMEDICINE AND PHARMACOTHERAPY, (1996) 50 (3-4) 158-62. Journal code: A59; 8213295. ISSN: 0753-3322. Pub. country: France. Language: English.

AB We have studied the purine nucleotide metabolism in the following cell lines: a), H9 (continuous human T-cell line) and H9/HTLV-III (H9 cell line, infected with ***RT*** + ***HIV*** -I virus); b), A3.01 (human lymphoblastoid cell line CD4+) and 8E51 (line A3.01 permanently transfected with ***RT*** - ***HIV*** -I virus). Purine metabolism was studied by evaluating the content of the most important ribonucleotides (AMP-GMP-IMP-NAD-ADP-GDP-ATP- ***GTP***) and their ratios. We determined several differences between the cell lines before and after viral infection. All nucleotides except triphosphates were reduced in H9/HTLV-III with respect to H9 cells; in 8E51, however, triphosphates were markedly reduced, while monophosphates increased with respect to A3.01 uninfected cells. Also the ratios exhibited different behaviors, for example the total adenine nucleotides total guanine nucleotides ratio (sigma A/sigma G) was enhanced in H9/HTLV-III cells with respect to H9 and unaltered in 8E51 with respect to A3.01 cells. We may conclude that the ***HIV*** -I virus strongly influences the purine nucleotide metabolism of the host cells and that the changes are different when induced either by ***RT*** + or ***RT*** - virus.

L43 ANSWER 33 OF 33 MEDLINE
87258188 Document Number: 87258188. PubMed ID: 2439916. Site-specific mutagenesis of AIDS virus ***reverse*** ***transcriptase*** . Larder B A; Purifoy D J; Powell K L; Darby G. NATURE, (1987 Jun 25-Jul 1)

327 (6124) 716-7. Journal code: NSC; 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB ***Human*** ***immunodeficiency*** ***virus*** (***HIV***) is the causative agent of AIDS (acquired immune deficiency syndrome) a disease which poses a serious challenge to modern medicine. If we are to conquer this disease we will need a protective vaccine or effective drugs able to block the life cycle of the virus. An early stage in the invasion of the host cell is the conversion of the RNA genome of the virus to a double-stranded DNA intermediate which subsequently becomes integrated into the host cell chromosome. The enzyme ***reverse*** ***transcriptase*** is crucial in this process and is thus an obvious chemotherapeutic target. In this study we have used site-directed mutagenesis of this enzyme expressed in Escherichia coli to reveal several important functional regions of the protein including putative components of the triphosphate binding site and ***pyrophosphate*** exchange sites.

L43 ANSWER 31 OF 33 MEDLINE
89008475 Document Number: 89008475. PubMed ID: 2459125. Studies on the mechanism of ***human*** ***immunodeficiency*** ***virus*** ***reverse*** ***transcriptase*** . Steady-state kinetics, processivity, and polynucleotide inhibition. Majumdar C; Abbotts J; Broder S; Wilson S H. (Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland 20892.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1988 Oct 25) 263 (30) 15657-65. Journal code: HIV; 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB A study of steady-state kinetics of polymerization by purified ***human*** ***immunodeficiency*** ***virus*** DNA polymerase (***reverse*** ***transcriptase***) has been conducted. DNA synthesis was examined using a system of poly(rA) as template, oligo(dT) as primer, and dTTP as nucleotide substrate. The substrate initial velocity patterns point to an ordered mechanism with template-primer adding first. Product inhibition kinetics with either ***pyrophosphate*** or phosphonoformate are consistent with this mechanism. The ***human*** ***immunodeficiency*** ***virus*** ***reverse*** ***transcriptase*** acts processively in this replication system, but exhibits some probability of terminating after each dTMP addition to the nascent chain. The probability of terminating was approximately 20-fold higher after the first dTMP addition than after subsequent additions. With this information on the mode of polymerization, appropriate kinetic models and steady-state rate equations are discussed. In further studies, we found that a heterologous polynucleotide, poly(rC), is a potent inhibitor of the enzyme. The pattern of this inhibition is uncompetitive against template-primer, suggesting that interaction with free enzyme is not the mechanism of the inhibition.

L43 ANSWER 22 OF 33 MEDLINE
92246996 Document Number: 92246996. PubMed ID: 1374247. Contribution of the p51 subunit of ***HIV*** -1 ***reverse*** ***transcriptase*** to enzyme processivity. Huang S C; Smith J R; Moen L K. (Department of Chemistry and Biochemistry, Old Dominion University, Norfolk, Virginia 23529.) BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1992 Apr 30) 184 (2) 986-92. Journal code: 9Y8; 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB ***Human*** ***immunodeficiency*** ***virus*** Type I ***reverse*** ***transcriptase*** is active as either the homodimer (p66/p66) or the heterodimer (p66/p51). Purified recombinant p66 and p51 expressed in yeast were reconstituted in the presence of 60 mM sodium

pyrophosphate to enhance dimer formation. Comparison of the processivity of these two active reconstituted forms shows that the heterodimer is more processive than the homodimer with a cycle almost twice as long as judged by assays utilizing poly (U,G) as a challenger to primer-template. Binding assays demonstrated that the heterodimer has a higher affinity for primer-template than the homodimer and that the p51 subunit has an affinity equal to that of the heterodimer. These results suggest that the p51 subunit functions to increase processivity in the heterodimer.

L43 ANSWER 7 OF 33 MEDLINE
2000193797 Document Number: 20193797. PubMed ID: 10729133. The M184V mutation in the ***reverse*** ***transcriptase*** of ***human*** ***immunodeficiency*** ***virus*** type 1 impairs rescue of chain-terminated DNA synthesis. Gotte M; Arion D; Parniak M A; Wainberg M A. (McGill University AIDS Centre, Lady Davis Institute-Jewish General Hospital, Montreal, Quebec, Canada.) JOURNAL OF VIROLOGY, (2000 Apr) 74 (8) 3579-85. Journal code: KCV; 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Nucleoside analog chain terminators such as 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxy-3'-thiacytidine (3TC) represent an important class of drugs that are used in the clinic to inhibit the ***reverse*** ***transcriptase*** (***RT***) of ***human*** ***immunodeficiency*** ***virus*** type 1. Recent data have suggested that mutant enzymes associated with AZT resistance are capable of removing the chain-terminating residue with much greater efficiency than wild-type ***RT*** and this may, in turn, facilitate rescue of DNA synthesis; these experiments were performed using physiological concentrations of ***pyrophosphate*** or nucleoside triphosphates, respectively. The present study demonstrates that the M184V mutation, which confers high-level resistance to 3TC, can severely compromise the removal of chain-terminating nucleotides. Pyrophosphorolysis on 3TC-terminated primer strands was not detectable with M184V-containing, as opposed to wild-type, ***RT*** , and rescue of AZT-terminated DNA synthesis was significantly decreased with the former enzyme. Thus, mutated RTs associated with resistance to AZT and 3TC possess opposing, and therefore incompatible, phenotypes in this regard. These results are consistent with tissue culture and clinical data showing sustained antiviral effects of AZT in the context of viruses that contain the M184V mutation in the ***RT*** -encoding gene.

L43 ANSWER 8 OF 33 MEDLINE
1999060068 Document Number: 99060068. PubMed ID: 9843396. Phenotypic mechanism of ***HIV*** -1 resistance to 3'-azido-3'-deoxythymidine (AZT): increased polymerization processivity and enhanced sensitivity to ***pyrophosphate*** of the mutant viral ***reverse*** ***transcriptase*** . Arion D; Kaushik N; McCormick S; Borkow G; Parniak M A. (Lady Davis Institute for Medical Research, McGill University AIDS Centre, Sir Mortimer B. Davis-Jewish General Hospital, Montreal, Quebec, Canada.) BIOCHEMISTRY, (1998 Nov 10) 37 (45) 15908-17. Journal code: A0G; 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The multiple mutations associated with high-level AZT resistance (D67N, K70R, T215F, K219Q) arise in two separate subdomains of the viral ***reverse*** ***transcriptase*** (***RT***), suggesting that these mutations may contribute differently to overall resistance. We compared wild-type ***RT*** with the D67N/K70R/T215F/K219Q, D67N/K70R, and T215F/K219Q mutant enzymes. The D67N/K70R/T215F/K219Q mutant showed increased DNA polymerase processivity; this resulted from decreased

template/primer dissociation from ***RT*** , and was due to the T215F/K219Q mutations. The D67N/K70R/T215F/K219Q mutant was less sensitive to AZTTP (IC50 approximately 300 nM) than wt ***RT*** (IC50 approximately 100 nM) in the presence of 0.5 mM ***pyrophosphate*** . This change in ***pyrophosphate*** -mediated sensitivity of the mutant enzyme was selective for AZTTP, since similar Km values for TTP and inhibition by ddCTP and ddGTP were noted with wt and mutant ***RT*** in the absence or in the presence of ***pyrophosphate*** . The D67N/K70R/T215F/K219Q mutant showed an increased rate of pyrophosphorolysis (the reverse reaction of DNA synthesis) of chain-terminated DNA; this enhanced pyrophosphorolysis was due to the D67N/K70R mutations. However, the processivity of pyrophosphorolysis was similar for the wild-type and mutant enzymes. We propose that ***HIV*** -1 resistance to AZT results from the selectively decreased binding of AZTTP and the increased pyrophosphorolytic cleavage of chain-terminated viral DNA by the mutant ***RT*** at physiological ***pyrophosphate*** levels, resulting in a net decrease in chain termination. The increased processivity of viral DNA synthesis may be important to enable facile ***HIV*** replication in the presence of AZT, by compensating for the increased reverse reaction rate.

L44 ANSWER 1 OF 1 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
AN 1997-200906 [18] WPIDS
DNN N1997-166106 DNC C1997-064177
TI Measuring DNA polymerising enzyme activity in body fluids, cells or virus infected cultures - comprises measurement of nucleotide incorporated with polymer synthesised by reaction of enzyme and primer/template prod. complex, useful for diagnosis of tumours, AIDS, leukaemia or herpes.
DC B04 C06 D16 S03
IN GRONOWITZ, J; KALLANDER, C; ***LENNERSTRAND, J*** ; KAELLANDER, C
PA (GRON-I) GRONOWITZ J; (KALL-I) KALLANDER C; (CAVI-N) CAVIDI TECH AB; (KAEL-I) KAELLANDER C
CYC 72
PI SE 9502765 A 19970205 (199718)* 32p
WO 9806873 A1 19980219 (199815)#
RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG
W: AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN
AU 9672325 A 19980306 (199830)#
EP 856066 A1 19980805 (199835) EN
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LT LU LV MC NL PT SE SI
US 6132995 A 20001017 (200054)#
ADT SE 9502765 A SE 1995-2765 19950804; WO 9806873 A1 WO 1996-SE990 19960812; AU 9672325 A AU 1996-72325 19960812; EP 856066 A1 EP 1996-933690 19960812, WO 1996-SE990 19960812; US 6132995 A WO 1996-SE990 19960812, US 1998-51084 19980413
FDT AU 9672325 A Based on WO 9806873; EP 856066 A1 Based on WO 9806873; US 6132995 A Based on WO 9806873
PRAI SE 1995-2765 19950804; WO 1996-SE990 19960812; AU 1996-72325 19960812; US 1998-51084 19980413

AB SE 9502765 A UPAB: 19990224
Method for determining the activity of a nucleotide-polymerising enzyme in a sample comprises: (a) capturing the enzyme with a monoclonal antibody (MAb) immobilised on a fixed carrier (the MAb binds the enzyme without significantly affecting its activity); (b) removing impurities and/or disturbance factors; (c) starting nucleotide polymerisation by adding a reaction soln. contg. a primer/template construction and nucleotide substrate; (d) providing reaction conditions to favour permanent association between the enzyme and primer/template prod. complex; (e) washing the nucleotide substrate, primer/template and reaction soln. to remove freshly synthesised polymer; (f) measuring the amt. of nucleotide incorporated with the polymer by standard techniques, and (g) determining enzyme activity.

USE - The method is used for measuring the activity of certain DNA-polymerising enzymes, e.g. HIV1 RT-polymerase, herpes simplex DNA-polymerase, retro RT, DNA-virus-polymerases, or cellular gamma - or delta -polymerase, in human or animal body fluids, cell samples or virus-infected culture sample. This method is also used for isoenzyme characterisation and for the diagnosis and prognosis of tumours or illnesses caused by or associated with virus infections, e.g. AIDS, T-cell leukaemia or herpes.

ADVANTAGE - Background disturbance due to cellular polymerase is eliminated.lo

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